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Appendix 7

HOWARD HUGHES MEDICAL INSTITUTE

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HHMI News

Building a Better Mouse Model of Lung Cancer

"We believe that this new mouse model more accurately mimics tumor development in humans, which may lead to new insights into the genetic changes that occur in human disease," said HHMI investigator Tyler Jacks.

April 26, 2001— Researchers have genetically engineered cancer-prone mice that carry cells that switch on a cancer-causing gene spontaneously, generating lung and other cancers much like humans do. The scientists believe that the technique for generating the mice will be widely applicable and may be used to model many kinds of human cancers in mice.

In an article published in the April 26, 2001, issue of the journal *Nature*, Howard Hughes Medical Institute (HHMI) investigator Tyler Jacks of the Massachusetts Institute of Technology (MIT) and his colleagues reported that they used a "hit-and-run" approach to producing gene alterations in mice whose cells harbor an inactivated form of the *K-ras* cancer gene, or oncogene. Co-authors of the paper include Leisa Johnson, who is now at Onyx Pharmaceuticals, and colleagues at MIT, as well as collaborators at Tufts University Schools of Medicine and Veterinary Medicine and the Dana-Farber Cancer Institute.

Mutations in *K-ras* are highly prevalent in human cancers, occurring in 90 percent of pancreatic tumors, 50 percent of colon tumors and 30 percent of non-small cell lung cancers. Previous mouse models of these forms of cancer have been informative, said Jacks, but they have not accurately recapitulated the kind of spontaneous mutations that characterize cancers involving *K-ras*. Some of the obstacles have been technical in nature. For example, said Jacks, creating a mouse embryo with cells that contain mutated *K-ras*, which is a dominant mutation, would either be severely damaging or lethal to the embryo.

"Researchers have tried to overcome the problem of dominance by making transgenic mouse strains that only express the dominant oncogene in cells of a given tissue," said Jacks. "The problem is that neither embryonic insertion nor such tissue-specific transgenic mice recapitulate what one finds in normal human cancer, where individual cells acquire an oncogenic mutation, but they are otherwise surrounded by normal cells.

"Our strategy was to create a kind of genetic Trojan horse," said Jacks. They did this by introducing latent *K-ras* genes into mice. The genes were inactivated because they had duplicated segments of DNA that prevented the genes from activating themselves.

"Then as the mice grew, individual cells underwent rare spontaneous, sporadic recombination events that deleted one copy of the duplicated sequence, such that the *K-ras* gene was activated and began to initiate tumor development." The technique was based on the hit-and-run gene-targeting technology developed by HHMI investigator Allan Bradley at Baylor College of Medicine. The two-part technique consists of "hitting" cells with an inserted mutated gene and then allowing the recombination event to "run,"

activating the inserted gene.

The mice carrying the mutated *K-ras* gene system that Jacks and his colleagues inserted did, indeed, develop several types of tumors, including skin cancers and lymphomas. The animals also showed high incidence of early onset lung tumors.

"We believe that this new model more accurately mimics tumor development in humans, which may lead to new insights into the genetic changes that occur in human disease," said Jacks. "We can isolate tumors at various stages of progression, and thus, discover genes and processes that accompany tumorigenesis."

Furthermore, said Jacks, "the idea of creating a silent, or latent, version of an oncogene could be applied to any dominant mutation that you want to activate only sporadically in a small percentage of cells."

In an additional experiment, the scientists also produced *K-ras* mice with a mutant form of *p53*, a tumor suppressor gene whose malfunction is known to spur the progression of *K-ras*-based human lung cancers. These double-mutant mice showed reduced lifespan when compared to mice with either *p53* or *K-ras* mutation alone. This further demonstrates the relevance of their mouse model to human lung cancers, said Jacks.

The only significant limitation to the technique, said Jacks, is that the lung tumors arise so rapidly that the mice are overcome by tumors before there is a chance to observe the process of metastasis, the spread of lung cancer to other tissues. However, Jacks and his colleagues are developing a second-generation mouse model that will allow them to control and monitor the "run" part of the hit-and-run system in order to study metastasis more effectively.

Although Jacks's laboratory will focus mainly on lung cancer, their studies of the mice have already offered new insights into other *K-ras*-related cancers. "These mice don't develop colon tumors, although we might expect them to because *K-ras* is mutated in human colon cancer at high frequency," said Jacks. "This tells us that the order of mutation matters in colon cancer, which is caused by multiple mutations." The idea that the order of mutation is important is an argument that has been advanced by HHMI investigator Bert Vogelstein at Johns Hopkins University, based on his studies of human colon cancers, said Jacks.

Particularly promising, said Jacks, is the immediate potential for using the new mouse model to test both chemotherapies and chemopreventives for lung cancer. "These animals develop early-onset lung cancer and even earlier onset lung lesions," he said. "This rapid development, and the fact that these lesions develop spontaneously mean that animals can be tested for their response to drugs very quickly, and without having to be treated first with carcinogens, as in past testing regimes. And, since mutations in *K-ras* occur with high frequency in this type of lung cancer, these mice should respond to therapeutics aimed at *K-ras*." Also, added Jacks, strategies to prevent such lung cancers, which formerly required expensive, long-term clinical trials in humans, could be tested quite readily using the new mouse strain.

Photo: Stanley Rowin

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